Low threshold levels of ultraviolet-B in a background of photosynthetically active radiation trigger rapid degradation of the D2 protein of photosystem-II

Marcel A.K. Jansen[1,2,*], Victor Gaba[3],
Bruce M. Greenberg[4], Autar K. Mattoo[5] and
Marvin Edelman[1]
[1]Department of Plant Genetics, Weizmann Institute of
Science, Rehovot 76100, Israel,
[2]Department of Plant Physiology, Wageningen
Agricultural University, Arboretumlaan 4,
6703 BD Wageningen, The Netherlands,
[3]Department of Virology, ARO Volcani Centre, P.O.B. 6,
Beit Dagan 50250, Israel,
[4]Department of Biology, University of Waterloo,
Waterloo, Ont. N2L-3GI, Canada, and
[5]Plant Molecular Biology Laboratory, USDA/ARS,
Beltsville Agricultural Research Centre, Beltsville,
MD 20705, USA.

Summary

The photosystem II reaction centre has at its core a heterodimer made up of two proteins, D1 and D2. The D1 protein is known to be rapidly degraded by photosynthetically active radiation while the D2 protein is relatively stable. This paper reports that when the aquatic higher plant, *Spirodela* was exposed to ultraviolet-B radiation, D2 degradation accelerated markedly and half life times approached those of the D1 protein. Moreover, in the presence of an environmentally relevant background of photosynthetically active radiation, low fluxes of ultraviolet-B (but not ultraviolet-A) radiation synergistically stimulated degradation of the D2 protein within functional reaction centres. Thus, above a critical threshold, ultraviolet-B specifically targets the D1–D2 heterodimer for accelerated degradation.

Introduction

An increasing level of ultraviolet-B irradiation (UV-B ; 280–320 nm) is currently penetrating the biosphere (Kerr and McElroy, 1993). Exposure of plants to high UV-B levels can lead to alterations in growth and development, transpiration and photosynthesis (Teramura and Sullivan, 1994; Tevini, 1993). Experimental evidence points to photosystem II (PSII) (Barnes *et al.*, 1988; Bornman, 1989; Melis *et al.*, 1992; Noorudeen and Kulandaivelu, 1982; Renger *et al.*, 1989; Strid *et al.*, 1994) as the main target for UV-B damage in photosynthesis.

PSII is a protein–pigment complex consisting of a reaction centre, a water splitting system, and a light-harvesting chlorophyll protein complex (LHCII) (Mattoo *et al.*, 1989; Rochaix and Erickson, 1988). The PSII reaction centre contains a heterodimer core made up of the D1 and D2 proteins (Marder *et al.*, 1987; Nanba and Satoh, 1987; Trebst, 1986). These proteins bind chlorophyll, pheophytin, carotenoid and plastoquinone molecules, and are pivotal in catalysing light-dependent oxygen evolution and photophosphorylation-coupled linear electron flow (Mattoo *et al.*, 1989; Rochaix and Erickson, 1988). The D1 protein also provides the binding environment for several commercial herbicides (Bowyer *et al.*, 1991; Jansen *et al.*, 1993a; Trebst *et al.*, 1988). Structural changes in the D1–D2 heterodimer were implicated in UV-B-induced inhibition of the oxidizing and reducing activities of PSII (Melis *et al.*, 1992; Renger *et al.*, 1989).

Despite functional and structural similarities between the D1 and D2 proteins, the two differ considerably in their *in vivo* rate of turnover. D1 is degraded rapidly under photosynthetically active radiation (PAR; 400–700 nm) (Gounaris *et al.*, 1987; Mattoo *et al.*, 1984; Mullet *et al.*, 1990; Ohad *et al.*, 1990) while D2 is stable (Gounaris *et al.*, 1987; Greenberg *et al.*, 1987). Rapid degradation of D2 *in vivo* has only previously been reported following strong, non-physiological irradiation with PAR (Schuster *et al.*, 1988). In this study we report that D2 as well as D1 degrade rapidly when plants are UV-B irradiated. Moreover, in mixed-light experiments, using environmentally relevant threshold rates of UV-B combined with realistic backgrounds of PAR, the D1–D2 heterodimer rapidly and specifically degrades. UV-A irradiation (320–400 nm) is ineffective in driving this mixed-light reaction. Thus, PS-II reaction centre stability may be limited by sensitization of UV-B-driven D2 protein degradation.

Results and discussion

The D2 and D1 proteins are preferentially degraded under UV-B irradiance

The D2 protein is relatively stable—*vis-à-vis* the D1 protein—under physiological intensities of PAR (Gounaris *et al.*, 1987; Greenberg *et al.*, 1987; Mullet *et al.*, 1990;

Ohad *et al.*, 1990). We now find that D2 protein degradation is strongly enhanced by UV-B irradiation. The results of pulse-chase experiments using *Spirodela* plants are shown in Figure 1(a). Among the radiolabelled membrane proteins bands seen on the SDS–polyacrylamide gel, the D1 and D2 proteins were both rapidly chased in the UV-B, only the D1 protein was rapidly chased in PAR, and both proteins were stable in the dark. Quantification of data from a number of such experiments indicated that the ratio of half life times of D1 : D2 increased from 0.2 in PAR to 0.8 in the UV-B.

Degradation as a function of UV-B photon flux (300 nm; 3–23 μmol m^{-2} sec^{-1}) was compared for several chloroplast proteins. Degradation of the D1 and D2 proteins accelerated significantly with UV-B irradiance, while the chlorophyll-binding PSII-core proteins (CP43 and CP47), the proteins of the PSII light-harvesting chlorophyll *a/b* complex (LHCII), and the large subunit of rubisco (LS) were stable (Figure 1b). Although rubisco responds to UV-B irradiation (Vu *et al.*, 1982; Wilson *et al.*, 1995), it is primarily activity and not protein amount which decreases after irradiation (Jordan *et al.*, 1992). Thus, UV-B-driven degradation of the D1 and D2 proteins is specific and photon-fluence rate-dependent. These findings raise the possibility that the D1–D2 heterodimer, rather than just the D1 protein (Greenberg *et al.*, 1989), is a target of UV-B damage to PSII.

Photosynthetically active radiation strongly promotes UV-B-driven D2 degradation

Most UV-B-mediated effects on plants are strongly dampened (Teramura and Sullivan, 1994; Teramura *et al.*, 1980) by the high-background intensities of PAR which prevail in nature (Reid *et al.*, 1991). This is due to the effects of PAR on physiological and morphological processes which, in turn, determine UV-B sensitivity (Tevini and Teramura, 1989). Oxygen evolution in *Spirodela*, determined photoacoustically, saturates at approximately 1000 μmol m^{-2} sec^{-1} PAR (Jansen, 1993). In an attempt to evaluate the environmental relevance of UV-B-driven D2 degradation, *Spirodela* plants were simultaneously exposed to a photosynthesis-saturating photon flux of 1000 μmol m^{-2} sec^{-1} PAR and various fluence rates of UV-B irradiation. Above a threshold level of UV-B irradiance, the plants showed sharply accelerated D2 degradation along with a stimulation of D1 degradation: a fluence rate of more than 0.25 μmol m^{-2} sec^{-1} UV-B was required to accelerate degradation, while saturation of the effect was attained by 1.0 μmol m^{-2} sec^{-1} (Figure 2). The acceleration of D2 protein degradation was clearly a synergistic response to PAR plus UV-B, being greater than the additive value of the component radiation's applied alone (Figure 2). D1 degradation seemingly saturates at approximately 1000 μmol m^{-2} sec^{-1} PAR (Jansen, 1993). The additional
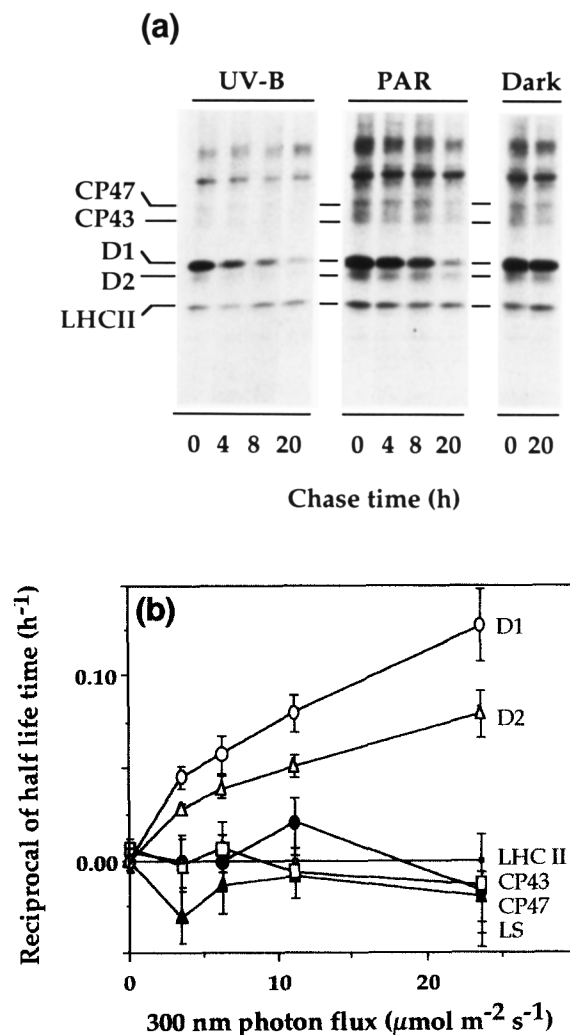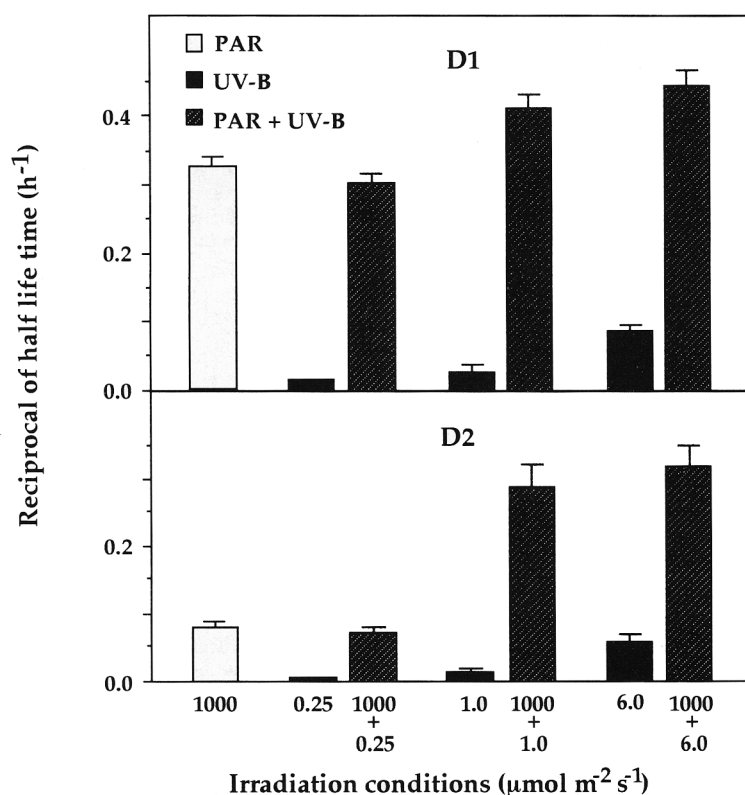

Figure 1. Degradation of the D1 and D2 proteins.
(a) UV-B-induced degradation of the D1 and D2 proteins. *S. oligorrhiza* plants were radiolabelled with [^{35}S]methionine for 2 h under 25 μmol m^{-2} sec^{-1} of PAR and chased in 1 mM methionine for the times indicated below the gel. The following radiation conditions applied during the chase: UV-B, 6 μmol m^{-2} sec^{-1}; PAR, 6 μmol m^{-2} sec^{-1}; or darkness. Plants were then homogenized and their membrane proteins fractionated by SDS–PAGE (10–20% gradients). Gels were loaded on an equal protein basis (10 μg lane^{-1}) and the proteins were detected by autoradiography. Positions of the D1, D2, light-harvesting chlorophyll *a/b* protein of PSII (LHCII), and chlorophyll-proteins CP43 and CP47 are indicated. Proteins were identified as described previously (Callahan *et al.*, 1989).
(b) Specific D2 and D1 degradation *in vivo* as a function of 300 nm photon flux. Spirodela plants were pulse-labelled as described, rinsed, and then incubated under UV-B radiation. Proteins were isolated, fractionated on SDS–PAGE and protein degradation kinetics determined (Greenberg *et al.*, 1987). The straight baseline represents LHCII ($\square$), to which D1 ($\bigcirc$), D2 ($\triangle$), CP43 ($\square$), CP47 ($\bullet$) and LS ($\blacktriangle$) were normalized (Greenberg *et al.*, 1989). Values represent averaged data from several experiments: D1 and D2, n = 14–21; CP43, CP47 and the large subunit of Rubisco (LS), n = 6–12. Standard errors are shown.

enhancement of D1 degradation under mixtures of UV-B and PAR, also indicates an interaction between the two wavebands used. Thus, an environmentally relevant back-

Figure 2. Degradation of the D1 and D2 proteins in mixtures of PAR and UV-B irradiation. Protein degradation was determined in *Spirodela*, radiolabelled and prepared as described, and chased under either PAR (1000 μmol m^{-2} sec^{-1}), UV-B irradiation, or a mixture of both. Values represent averaged data from several experiments; $n = 6$–18. Standard errors are shown.

ground of PAR strongly promoted, rather than dampened, UV-B-driven D2 degradation. Indeed, this synergism is the major factor in D1–D2 heterodimer degradation, being more severe than the effect of UV-B or PAR alone. It may reflect the interaction of multiple photosensitizers (Greenberg *et al.*, 1989) in the pathway leading to protein degradation. These results suggest a general need to reassess cellular targets of UV-B irradiation damage in photosynthetic organisms under appropriate mixtures of PAR and UV-B radiation.

UV-A radiation is ineffective in driving D2 degradation

The level of UV-A irradiance (320–400 nm) in the biosphere is considerably higher (5 to 40-fold) than that of UV-B (Reid *et al.*, 1991; Tevini, 1993). If D2 degradation is also promoted by a mixture of UV-A radiation and PAR, then any effect of increased UV-B irradiation on D2 degradation will probably be irrelevant. In this regard, exposure of *Spirodela* plants to a combination of 6 μmol m^{-2} sec^{-1} of 350 nm radiation and 100 μmol m^{-2} sec^{-1} of PAR did not result in kinetics of D2 or D1 degradation significantly different than those obtained with 100 μmol m^{-2} sec^{-1} of PAR alone (Figure 3). This level of PAR was chosen to maintain a ratio of PAR to UV-A approximating that found in nature (100 : 5–10) (see Reid *et al.*, 1991; Tevini, 1993). Thus, in the presence of a background of PAR, it is specifically UV-B irradiation which

leads to enhanced degradation of both the D1 and D2 proteins.

The synergistic stimulation of D1–D2 heterodimer degradation by mixtures of UV-B and PAR may reflect the interaction of multiple photosensitizers (Greenberg *et al.*, 1989). Two alternative mechanisms are considered. Based on analysis of the radiance spectra for D1 protein degradation and inhibitor studies, D1-bound plastosemiquinone-B was previously proposed as a photosensitizer for UV-B-mediated D1 protein degradation (Greenberg *et al.*, 1989; Jansen *et al.*, 1993b). We speculate that accelerated degradation of the D2 and D1 proteins by mixtures of UV-B and PAR arises due to the production of plastosemiquinone anions (Q_A^- and Q_B^-) which necessarily accompany PAR-driven electron flow. It is expected that similar amounts of Q_B^- will be present under low and high fluences of PAR. Consistent with this, we have found enhancement of D1 degradation under low (Jansen *et al.*, 1993b) and high (cf. Figure 2) levels of PAR when combined with a low fluence rate of UV-B.

One must also seriously consider the alternative possibility that aromatic amino acid residues play a role as UV photosensitizer (Kim *et al.*, 1992). Tyrosine radicals (e.g. Tyr Z$^+$) generated during photosynthetic electron flow may function as photosensitizers for UV-B-induced D1 and/or D2 degradation. The absorbance difference spectrum of Tyr Z$^+$–Z has been precisely measured (Dekker *et al.*,
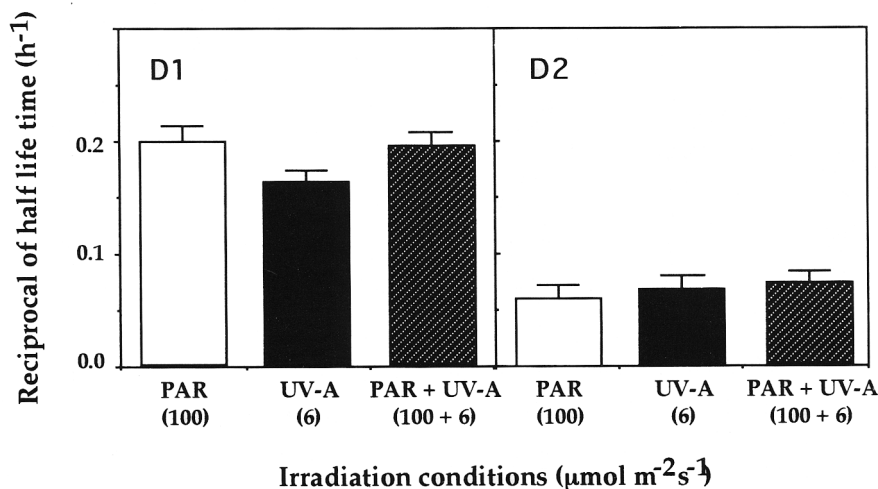
Figure 3. The effects of UV-A irradiation on D1 and D2 protein degradation in a background of PAR.
Protein degradation was determined in *Spirodela* as described. PAR was 100 μmol m^{-2} sec^{-1}; UV-A radiation was 6 μmol m^{-2} sec^{-1}; and PAR + UV-A was a mixture of the two. Values represent averaged data from several experiments; n = 12–16. Standard errors are shown.
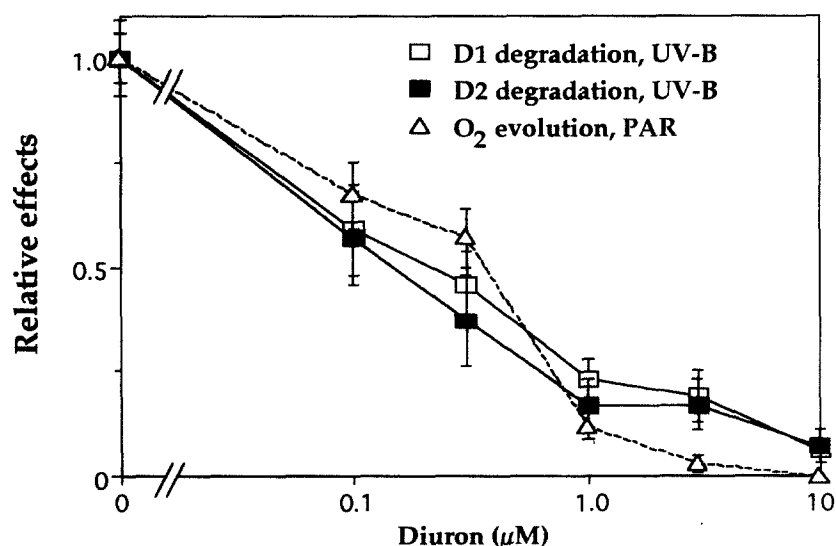
Figure 4. The relative effects of diuron on UV-B-driven degradation of the D2 and D1 proteins.
Protein degradation was determined in *Spirodela* plants following pulse-chase experiments as in the legend to Figure 1. Radiolabelled plants were chased in the presence and absence of 10 μM diuron under 6 μmol m^{-2} sec^{-1} UV-B radiation (300 nm). Relative quantum yields of oxygen evolution were measured *in vivo* in visible light, by photoacoustic spectroscopy (Jansen *et al.*, 1993a). For all curves, n = 15. Standard errors are shown.

1984), and contains a peak around 300 nm. However, the absorbance difference spectrum deviates in the UV-C region from the spectra for D1 (Greenberg *et al.*, 1989; Jansen *et al.*, 1993b) and D2 (Jansen, 1993) degradation. Thus, it is difficult to draw positive conclusions about the involvement of tyrosine radicals in the degradation of the D1 and D2 proteins at the present time.

UV-B-driven D1 and D2 degradation are linked processes in oxygen-evolving reaction centres

To probe the physiological significance of UV-B-driven D1 and D2 degradation, we treated *Spirodela* plants with several concentrations of the PSII inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), and measured concurrent inhibition of PAR-driven oxygen evolution (taken as an indicator of active PSII reaction centres) and UV-B-driven D1–D2 degradation. Degradation of the D1 and D2

proteins was impeded linearly and in parallel, as was oxygen evolution (Figure 4). The correlation coefficient (r^2) between the curves for D1 and D2 was 0.99; between D1 and O_2 evolution, 0.97; and between D2 and O_2 evolution, 0.96. These data indicate a close linkage among the three processes and reveal that degradation observed in UV-B is that of the D1 and D2 proteins in functionally-assembled PSII reaction centres.

Diuron is well known to displace Q_B^- from its binding site on the D1 protein (Bowyer *et al.*, 1991; Jansen *et al.*, 1993a; Trebst *et al.*, 1988), thereby blocking PSII electron flow, oxygen evolution and D1 degradation (Jansen *et al.*, 1993a). However, there is no evidence that it displaces Q_A^- from its niche on the D2 protein. We interpret our results as indicating that catabolism of the two proteins is linked. These data (Figure 4) are in line with trypsinization (Trebst 1991; Trebst *et al.*, 1988) and mutagenesis (Kless *et al.*, 1993) experiments indicating a linkage between D1 and D2

in the vicinity of the plastoquinone-binding environments. Indeed, decreasing the level of the D1 protein using chloramphenicol (Christopher and Mullet, 1994) or mutagenesis (Vermaas and Ikeuchi, 1991) results in a destabilization of the D2 protein. Thus, diuron might be inhibiting D2 degradation because D1 catabolism has been compromised.

Environmental significance

In addition to our *in vivo* studies, D2 protein degradation has also recently been observed in isolated reaction centres exposed to UV-B irradiation (Friso *et al.*, 1994). It is not presently known if D1–D2 heterodimer degradation is significant in the field under current UV-B levels. Sunlight contains approximately 7.5 $\mu mol\ m^{-2}\ sec^{-1}$ of UV-B irradiation at a photon flux density (as in Figure 2) of 1000 $\mu mol\ m^{-2}\ sec^{-1}$ of PAR (Reid *et al.*, 1991). However, it is difficult to extrapolate from the UV-B threshold value (less than 1.0 $\mu mol\ m^{-2}\ sec^{-1}$; Figure 2) for heterodimer degradation obtained with laboratory-grown *Spirodela* plants to conditions outdoors. In nature, selective absorption by UV-absorbing pigments in the epidermis and cuticle can severely (90–99%) diminish transmission of UV-B irradiance to the chloroplasts in the mesophyll (Caldwell *et al.*, 1983; DeLucia *et al.*, 1992). Moreover, some plants respond to increased UV-B irradiance by elevating their UV-B screening capacity (Reddy *et al.*, 1994; Robberecht and Caldwell, 1983; Teramura and Sullivan, 1994; Wellmann, 1975), thereby protecting PSII activity and stabilizing the D1 protein (Wilson and Greenberg, 1993b). These factors, along with screening from antenna chlorophylls (Greenberg *et al.*, 1989), may normally lower UV-B irradiance reaching PSII reaction centres to below the threshold for accelerated D1–D2 heterodimer degradation. However, as UV-B increases in the environment, this threshold may be more frequently realized, especially in UV-B sensitive species. Indeed, Booij *et al.* (1995) using UV-B-sensitive (cv. CNS) and tolerant (cv. Williams) soybean cultivars, have found that under PAR, kinetics of D1 and D2 degradation are similar for the two cultivars. However, under UV-B radiation, and especially in UV-B plus PAR, degradation of the D1–D2 heterodimer was significantly enhanced in cv. CNS compared with cv. Williams.

Experimental procedures

Spirodela oligorrhiza plants were grown photoautotrophically (Gaba *et al.*, 1987) under 25 $\mu mol\ m^{-2}\ sec^{-1}$ PAR provided by cool white fluorescent lamps. Fronds, covering a 9 cm petri dish, were pulse-labelled in 10 ml medium, containing 100 $\mu Ci\ [^{35}S]$methionine (800 mCi $mmol^{-1}$) for 2 h under 25 $\mu mol\ m^{-2}\ sec^{-1}$ PAR, rinsed, and chased for various periods of time in growth medium containing 1 mM non-radioactive methionine. The higher the fluence rates applied to the plant, the shorter the irradiation times employed during the chase. For example: 0.5, 1, and 2 h time

points were used for 1000 $\mu mol\ m^{-2}\ sec^{-1}$ PAR plus 6 $\mu mol\ m^{-2}\ sec^{-1}$ 300 nm mixed irradiation conditions; 2, 4 and 8 h points for 100 $\mu mol\ m^{-2}\ sec^{-1}$ PAR plus 6 $\mu mol\ m^{-2}\ sec^{-1}$ 350 nm mixed irradiation conditions, or for 12 and 23 $\mu mol\ m^{-2}\ sec^{-1}$ irradiation at 300 nm; while 4, 8 and 20 h points were employed for single irradiance experiments (UV-B, UV-A or PAR) at fluxes of 3 or 6 $\mu mol\ m^{-2}\ sec^{-1}$. In this way, all calculations of degradation kinetics were based on data points taken within the first two half lives of the D1 protein.

Chase periods were terminated by draining the medium and freezing the plants. Membrane proteins were solubilized, fractionated by SDS–PAGE, visualized by autoradiography and bands quantified by densitometry (Greenberg *et al.*, 1987). Peak heights of the D2 and D1 bands were normalized to LHCII peak height. The *in vivo* half life times were determined for each individual chase time point, by comparison with the 0 h time point, and assuming first-order kinetics (Greenberg *et al.*, 1989). The kinetics of degradation during the chase followed an exponential decay curve at all wavelengths tested. The reciprocal of the half life time was used (cf. Figures 1, 2, 3) as a measure of degradation rate. Oxygen evolution *in vivo* was determined in visible light by photoacoustic spectroscopy (Jansen *et al.*, 1993a).

Radiation sources during the chase periods were as follows: in the UV-B spectral range, a 300 nm photoreactor tube (Rayonet, Southern New England Ultraviolet Co., Branford, CT, USA) having a bandwidth of 40 nm at half maximum output, with 1.1% of the fluence at wavelengths less than 280 nm; in the UV-A spectral range, a Rayonet 350 nm photoreactor tube having a bandwidth of 30 nm at half maximum output; in the PAR range, a tungsten halogen projector with KG3 heat-absorbing glass (Schott) and 2.5 cm water filter. Photon flux densities in the UV spectral range were measured with a UVX Digital Radiometer, using a UVX-31 sensor (peak 310 nm) designed to measure radiation from UV-B lamps, and a UVX-36 sensor (peak 360 nm) designed for UV-A lamps (UVP, inc. San Gabriel, CA, USA). For the PAR spectral range, irradiance was measured using a LI-189 quantum sensor (Li-Cor inc., Lincoln, NE, USA).

In mixed light experiments, the radiation sources were placed as directly over the plants as possible. Fluence rates were adjusted by changing the distance of the lamps to the plants. To allow comparison with the work of others, we have estimated the biologically effective UV-B radiation as the cross-sectional overlap between the generalized plant action spectrum normalized to 300 nm (Caldwell *et al.*, 1986) and the spectral output of the Rayonet 300 nm photoreactor tube (Wilson and Greenberg, 1993a). For 0.25, 1 and 6 $\mu mol\ m^{-2}\ sec^{-1}$ of radiation in the UV-B region, the values were 0.11, 0.43 and 2.6 $\mu mol\ m^{-2}\ sec^{-1}$, respectively. For 6 h of chase time, the overall doses at these corrected fluence rates would be 0.94, 3.8 and 22.7 $kJ\ m^{-2}$, respectively.

References

Barnes, P.W., Jordan, P.W., Gold, W.G., Flint, S.D. and Caldwell, M.M. (1988) Competition, morphology and canopy structure in

wheat (*Triticum aestivum* L.) and wild oat (Avena fatua L.) exposed to enhanced ultraviolet-B radiation. *Funct. Ecol.* **2**, 319–330.

Booij, I.S., Swegle, M., Dube, S., Edelman, M. and Mattoo, A.K. (1995) Photodegradation of D1-D2 PSII reaction center heterodimer. In *Xth Int. Photosynth. Congr. Proc.* (Mathis, P., ed.). Dordrecht: Kluwer Academic Publishers, pp. 487–490.

Bornman, J.F. (1989) Target sites of UV-B radiation in photosynthesis of higher plants. *Photochem. Photobiol.* **4**, 145–158.

Bowyer, J.R., Camilleri, P. and Vermaas, W.F.J. (1991) Photosystem II and its interactions with herbicides. In *Herbicides* (Baker, N.R. and Percival, M.P., eds). Amsterdam: Elsevier, pp. 27–85.

Caldwell, M.M., Robberecht, R. and Flint, S.D. (1983) Internal filters: prospects for UV-acclimation in higher plants. *Physiol. Plant.* **58**, 445–450.

Caldwell, M.M., Camp, L.B., Warner, C.W. and Flint, S.D. (1986) Action spectra and their key role in assessing biological consequences of solar UV-B radiation change. In *Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life*, NATO ASI, Volume G8 (Worrest, R.C. and Caldwell, M.M., eds). Berlin: Springer Verlag, pp. 87–111.

Callahan, F.E., Wergin, W.P., Nelson, N., Edelman, M. and Mattoo, A.K. (1989) Distribution of thylakoid proteins between stromal and granal lamellae in Spirodela. *Plant Physiol.* **91**, 629–635.

Christopher, D.A. and Mullet, J.E. (1994) Separate photosensory pathways co-regulate blue light/ultraviolet-A-activated psbD-psbC transcription and light-induced D2 and CP43 degradation in Barley (Hordeum vulgare) chloroplasts. *Plant Physiol.* **104**, 1119–1129.

Dekker, J.P., van Gorkom, H.J., Brok, M. and Ouwehand, L. (1984) Optical characterization of photosystem II electron donors. *Biochim. Biophys. Acta*, **764**, 301–309.

DeLucia, E.H., Day, T.A. and Vogelman, T.C. (1992) Ultraviolet-B and visible light penetration into needles of two species of subalpine conifers during foliar development. *Plant Cell Environ.* **15**, 921–929.

Friso, G., Barbato, R. , Giacometti, G.M. and Barber, J. (1994) Degradation of D2 protein due to UV-B irradiation of the reaction centre of photosystem II. *FEBS Lett.* **339**, 217–221.

Gaba, V., Marder, J.B., Greenberg, B.M., Mattoo, A.K. and Edelman, M. (1987) Degradation of the 32 kD herbicide binding protein in far red light. *Plant Physiol.* **84**, 348–352.

Gounaris, K., Pick, U. and Barber, J. (1987) Stoichiometry and turnover of photosystem II polypeptides. *FEBS Lett.* **211**, 94–98.

Greenberg, B.M., Gaba, V., Mattoo, A.K. and Edelman, M. (1987) Indentification of a primary *in vivo* degradation product of the rapidly-turning-over 32 kDa protein of photosystem II. *EMBO J.* **6**, 2865–2869.

Greenberg, B.M., Gaba, V., Canaani, O., Malkin, S., Mattoo, A.K. and Edelman, M. (1989) Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions. *Proc. Natl Acad. Sci. USA*, **86**, 6617–6620.

Jansen, M.A.K. (1993) *Light driven degradation of photosystem-II reaction centre proteins: Mechanism and environmental relevance.* Ph.D. Thesis. Rehovot, Israel: Weizmann Institute of Science.

Jansen, M.A.K., Mattoo, A.K., Malkin, S. and Edelman, M. (1993a) Direct demonstration of binding site competition *in vivo* between photosystem-II-inhibitors at the Q_B niche of the D1 protein. *Pestic. Biochem. Physiol.* **46**, 78–83.

Jansen, M.A.K., Gaba, V., Greenberg, B.M., Mattoo, A.K. and Edelman, M. (1993b) UV-B driven degradation of the D1 reaction

center protein of photosystem-II proceeds via plastosemiquinone. In *Photosynthetic Responses to the Environment* (Yamamoto, H.Y. and Smith, C.M., eds). Washington, DC: Plant Physiol. Soc., pp. 142–149.

Jordan, B.R., He, J., Chow, W.S. and Anderson, J.M. (1992) Changes in the mRNA levels and polypeptide subunits of ribulose-1,5-bisphosphate carboxylase in response to ultraviolet-B radiation. *Plant Cell Environ.* **15**, 91–98.

Kerr, J.B. and McElroy, C.T. (1993) Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science*, **262**, 1032–1034.

Kim, S.-T., Li, Y.F. and Sancar, A. (1992) The third chromophore of DNA photolyase: Trp-227 of *Escherichia coli* repairs thymine dimers by direct electron transfer. *Proc. Natl Acad. Sci. USA*, **89**, 900–904.

Kless, H., Oren-Shamir, M., Ohad, I., Edelman, M. and Vermaas, W. (1993) Protein modifications in the D2 protein of photosystem II affect properties of the Q_B/herbicide binding environment. *Z. Naturforsch.* **48c**, 185–190.

Marder, J.B., Chapman, D.J., Telfer, A., Nixon, P.J. and Barber, J. (1987) Identification of psbA and psbD gene products, D1 and D2, as reaction centre proteins of photosystem 2. *Plant Mol. Biol.* **9**, 325–333.

Mattoo, A.K., Hoffman-Falk, H., Marder, J.B. and Edelman, M. (1984) Regulation of protein metabolism: coupling of photosynthetic electron transport to *in vivo* degradation of the rapidly metabolized 32-kilodalton protein of chloroplast membranes. *Proc. Natl Acad. Sci USA*, **81**, 1380–1384.

Mattoo, A.K., Marder, J. and Edelman, M. (1989) Dynamics of the photosystem II reaction center. *Cell*, **56**, 241–246.

Melis, A., Nemson, J.A. and Harrison, M.A. (1992) Damage to functional components and partial degradation of photosystem II reaction centre proteins upon chloroplast exposure to ultraviolet-B radiation. *Biochim. Biophys. Acta*, **1100**, 312–320.

Mullet, J.E., Klein, P.G. and Klein, R.R. (1990) Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability. *Proc. Natl Acad. Sci USA*, **87**, 4038–4042.

Nanba, O. and Satoh, K. (1987) Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome b-559. *Proc. Natl Acad. Sci USA*, **84**, 109–112.

Noorudeen, A.M. and Kulandaivelu, G. (1982) On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B (UV-B) radiation. *Physiol. Plant.* **55**, 161–166.

Ohad, N., Amir-Shapira, D., Koike, H., Inoue, Y., Ohad, I. and Hirschberg, J. (1990) Amino acid substitutions in the D1 protein of photosystem II affect Q_B⁻ stabilization and accelerate turnover of D1. *Z. Naturforsch.* **45c**, 402–408.

Reddy, V.S., Goud, K.V., Sharma, R. and Reddy, A.R. (1994) Ultraviolet-B-responsive anthocyanin production in a rice cultivar is associated with a specific phase of phenylalanine ammonia lyase biosynthesis. *Plant Physiol.* **105**, 1059–1066.

Reid, D.M., Beallard, F.D. and Pharis, R.P. (1991) Environmental cues in plant growth and development. In *Plant Physiology: a Treatise, Growth and Development*, Volume 6 (Bidwell, R.G.S., ed.) San Diego: Academic Press, pp, 65–181.

Renger, G., Völker, M., Eckert, H.J., Fromme, R., Hohm-Veit, S. and Graeber, P. (1989) On the mechanism of photosystem II deterioration by UV-B irradiation. *Photochem. Photobiol.* **49**, 97–105.

Robberecht, R. and Caldwell, M.M. (1983) Protective mechanisms and acclimation to solar ultraviolet-B radiation in *Oenothera stricta*. *Plant Cell Environ.* **6**, 477–485.

Rochaix, J.-D. and Erickson, J. (1988) Function and assembly of photosystem II: genetic and molecular analysis. *Trends Biochem. Sci.* **13**, 56–59.

Schuster, G., Timberg, R. and Ohad, I. (1988) Turnover of thylakoid photosystem II proteins during photoinhibition of *Chlamydomonas reinhardtii. Eur. J. Biochem.* **177**, 403–410.

Strid, A., Chow, W.S. and Anderson, J.M. (1994) UV-B damage and protection at the molecular level in plants *Photosynth. Res.* **39**, 475–489.

Teramura, A.H. and Sullivan, J.H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynth. Res.* **39**, 463–473.

Teramura, A.H., Biggs, R.H. and Kossuth, S. (1980) Effects of ultraviolet-B increases on soybean. II. Interaction between ultraviolet-B and photosynthetically active radiation on net photosynthesis, dark respiration and transpiration. *Plant Physiol.* **65**, 483–488.

Tevini, M. (1993) Effects of enhanced UV-B radiation on terrestrial plants. In *UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms and Materials* (Tevini, M. ed.) Boca Raton, FL: Lewis Publishers, pp. 125–153.

Tevini, M. and Teramura, A.H. (1989) UV-B effects on terrestrial plants. *Photochem. Photobiol.* **50**, 479–487.

Trebst, A. (1986) The topology of the plastoquinone and herbicide binding peptides of photosystem II in the thylakoid membrane. *Z. Naturforsch.* **41c**, 240–245.

Trebst, A. (1991) A contact site between the two reaction centre

polypeptides of photosystem II is involved in photoinhibition. *Z. Naturforsch.* **46c**, 557–562.

Trebst, A., Depka, B., Kraft, B. and Johanningmeier, U. (1988) The Q_B site modulates the conformation of the photosystem II reaction center polypeptides. *Photosynth. Res.* **18**, 163–177.

Vermaas, W.F.J. and Ikeuchi, M. (1991) Photosystem II. In *The Photosynthetic Apparatus: Molecular Biology and Operation* (Bogorad, L. and Vasil, I.K., eds). San Diego: Academic Press, pp. 25–111.

Vu, C.V., Allen, L.H. and Garrard, L.A. (1982) Effects of supplemental UV-B radiation on primary photosynthetic carboxylating enzymes and soluble proteins in leaves of C3 and C4 crop plants. *Physiol. Plant.* **55**, 11–16.

Wellmann, E. (1975) UV dose-dependent induction of enzymes related to flavonoid biosynthesis in cell suspension cultures of Parsley. *FEBS Lett.* **51**, 105–107.

Wilson, M.I. and Greenberg, B.M. (1993a) Specificity and photomorphogenic nature of ultraviolet-B induced cotyledon curling in *Brassica napus* L. *Plant Physiol.* **102**, 671–677.

Wilson, M.I. and Greenberg, B.M. (1993b) Protection of the D1 photosystem II reaction centre protein from degradation in ultraviolet radiation following adaptation of *Brassica napus* L. to growth in ultraviolet-B. *Photochem. Photobiol.* **57**, 556–563.

Wilson, M.I., Ghosh, S., Gerhardt K.E., Holland, N., Babu, T. S., Edelman, M., Dumbroff, E.B. and Greenberg, B.M. (1995) *In vivo* photomodification of Ribulose-1,5-bisphosphate carboxylase/oxygenase holoenzyme by ultraviolet-B radiation. *Plant Physiol.* **109**, 221–229.